

The Tree Fern Highland Lace is a Cultivar of *Sphaeropteris cooperi*

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ABSTRACT.—The tree fern Highland Lace had an unusual introduction into cultivation almost thirty years ago in Eastern Australia and was initially identified as *Sphaeropteris tomentosissima* (Copel.) R.M.Tryon. Since then, it has been introduced to Europe and the US, and it remains a popular tree fern found in both public and private collections. We re-examined this fern, comparing it to a herbarium type specimen, and conclude that it is not *S. tomentosissima*, but is most likely a variant form of *Sphaeropteris cooperi* (F.V. Mueller) R.M.Tryon. Sequence analysis of chloroplast DNA [*rbcL*, *atpA* and *trnL* (UAA) intron] confirmed this species identification.

KEY WORDS.—Highland Lace, *Sphaeropteris tomentosissima*, *Sphaeropteris cooperi*, *Sphaeropteris excelsa*, tree ferns, chloroplast DNA sequence analysis, *rbcL*, *atpA*, *trnL* (UAA) intron

A distinctive tree fern with narrow pinnules and relatively small fronds appeared in Australian cultivation in the 1980s. It was a robust grower and its reduced pinnules imparted a lacy look to the leaves. Compared to most tree ferns, it was smaller, but it also seemed to bear more leaves in its crown. It originated as an unknown contaminant in a sporing pot at a wholesale nursery on the north coast of New South Wales, Australia. The late Rod Hill, an Australian tree fern enthusiast, made an attempt to identify the species and his closest match was *Sphaeropteris tomentosissima* (Copel.) R.M. Tryon, which grows in the highlands of west central New Guinea.

In the 1980s this plant spread among tree fern collectors and commercial growers in Australia and by the 1990s it was being grown in Europe and the United States. It is called either Highland Lace, New Guinea Treefern or *Sphaeropteris tomentosissima*. Its lacier appearance compared to other cultivated tree ferns has led to its high popularity, and it was awarded a first place and a trophy at the Los Angeles International Fern Society's annual Exotic Plant Show in 1997 and 2003 (Lois and Kurt Rossten, Huntington Beach, California).

Despite the enthusiasm for this new addition to the limited list of commercially available cultivated tree ferns, the identity of this fern was always a bit suspect, as noted by the question mark next to the species name on Rod Hill's former web site (Treeferns Down Under). We have re-examined this fern, and based on scale morphology and chloroplast DNA sequence analysis, conclude that it is actually a variant form of *Sphaeropteris cooperi* (F.V.Muell.) R.M.Tryon, rather than *S. tomentosissima*.

TABLE 1. Voucher information and GenBank accession numbers for tree ferns examined.

Species	Provenance	ID number	GenBank accession and reference
<i>S. tomentosissima</i>	Papua New Guinea	UC640117	
<i>S. tomentosissima</i>	Papua New Guinea	Conant 4581 (LSC)	Korall et al., 2006 <i>atpA</i> - AM176460 Korall et al., 2007 <i>rbcL</i> - AM177352 <i>trnL</i> intron - AM410304
<i>S. cooperi</i>	Highland Lace "Cultivated" Australia	Yansura 1 (UC)	<i>rbcL</i> - JN106035 <i>atpA</i> - JN106039 <i>trnL</i> intron - JN106036
<i>S. cooperi</i>	"Cultivated" Australia	Yansura 2 (UC)	<i>rbcL</i> - HM347350 <i>atpA</i> - JF690125 <i>trnL</i> intron - JF742607
<i>S. cooperi</i>	Flecker Botanical Gardens, Cairns Australia	Yansura 3 (UC)	<i>rbcL</i> - JN106038 <i>atpA</i> - JN106040 <i>trnL</i> intron - JN1060367

MATERIALS AND METHODS

The type specimen of *Sphaeropteris tomentosissima* (*Cyathea tomentosissima* Copel.) was examined and stipe scales were photographed at high resolution at the University and Jepson Herbaria at the University of California Berkeley (Brass 9116; UC 640117).

Leaf material for isolating chloroplast DNA was obtained from three sources: a cultivated plant of Highland Lace and a cultivated *Sphaeropteris cooperi*, both from the US (Hoshizaki's and Yansura's gardens); and four *S. cooperi* plants in the Flecker Botanical Gardens in Cairns, Australia. The latter four plants were carefully checked to be sure they had stipe scales consistent with *S. cooperi* as the garden had one plant labeled *Sphaeropteris excelsa* (Endlicher) R.M. Tryon, which could be confused with *S. cooperi* except for the scale differences. DNA was extracted from leaf material using the DNeasy Plant Mini Kit from QIAGEN (Valencia, California, USA), and the purified DNA was then used as a template to amplify three plastid loci (*rbcL*, *atpA*, *trnL* intron) using the polymerase chain reaction (PCR). The reaction was carried out with the appropriate set of primers and Cloned Pfu DNA polymerase from New England Biolabs (Ipswich, MA, USA) according to manufacturer's protocols. The PCR products were purified using the MinElute Reaction Cleanup kit from QIAGEN and then subjected to DNA sequencing on an ABI3730xl DNA Analyzer. All sequences (the four plants from the Flecker Botanical Garden had one common sequence) were deposited in GenBank (Table 1).

The beginning of the *rbcL* gene and the *atpB-rbcL* spacer were amplified with the primers *atpBR* or *atpBR1* and *RBCL158R*, the middle of the *rbcL* gene with primers *brun1* and *brun2*, and the 3' end as well as the *rbcL-accD* spacer with primers *RBCL1187F* and *ACCD887R*. The *atpA* gene was amplified by

TABLE 2. Primers used in amplification and sequencing. F = forward; R = reverse; S = sequencing.

Loci primer	Usage	Sequence (5'-3')	Reference
<i>rbcL</i> atpBR	F	TGAGCTTTGGCAATATTATTG	This study
<i>rbcL</i> atpBR1	F	TAATCTCTTGACCCGCTGGGTTAC	This study
<i>rbcL</i> RBCL158R	R S	AAGATTCCGCAGCTACTGCAGCTCC	Pryer, 2004
<i>rbcL</i> brun1	F S	CATTACCTTCACGAGCAAGGTCACGG	This study
<i>rbcL</i> RBCL1187F	F S	GGAACYTTGGGACATCCTTGG	Korall,2007
<i>rbcL</i> ACCD887R	R	TTATCACABCGMGCCCATTAATCC	Korall,2007
<i>rbcL</i> rbcf1	S	CCAAAATTGGGCTTATCTGCT	This study
<i>rbcL</i> rbcf2	S	CTAGCTTGGCCTTCTATTGCCG	This study
<i>atpA</i> ESATPF415F	F S	CARGTTCGACAGCAAGTYTCTCG	Schuettpelz,2006
<i>atpA</i> ESTRNR46F	R S	GTATAGGTTTCRARTCCTATTGGACG	Schuettpelz,2006
<i>atpA</i> atpAf	S	GACAGACTGGTAAAACAGCAGTAG	This study
<i>atpA</i> atpAr	S	TTGCCGGTCGAATGCCAGCATTAA	This study
<i>trnL</i> trn1	R S	ATTTGAACTGGTGACACGAGGATT	This study
<i>trnL</i> trn2	F S	CGAAATCGGTAGACGCTACGGACT	This study
<i>trnL</i> trn5	S	CTACCCTGTTCTGTTGGGGAT	This study
<i>trnL</i> trn6	S	TGCACGGGGGCTATTCCAACG	This study
<i>trnL</i> trn9	S	TGCAGTCTCTGTACCTATC	This study

PCR using the primers ESATPF415F and ESTRNR46F, and the *trnL* intron and flanking sequences were amplified with the primers trn1 and trn2. All primers used for PCR amplification and sequencing are listed in Table 2.

RESULTS

Our first indication that this tree fern might be misidentified was based on the stipe scales, which did not match the published description for *Sphaeropteris tomentosissima* (Holttum, 1963). Comparison with the type specimen reaffirmed that the two plants were very different in scales, leaf, and other details (Fig. 1 and 2A–C). Surprisingly, however, the stipe scales on Highland Lace closely match those of the commonly cultivated Australian tree

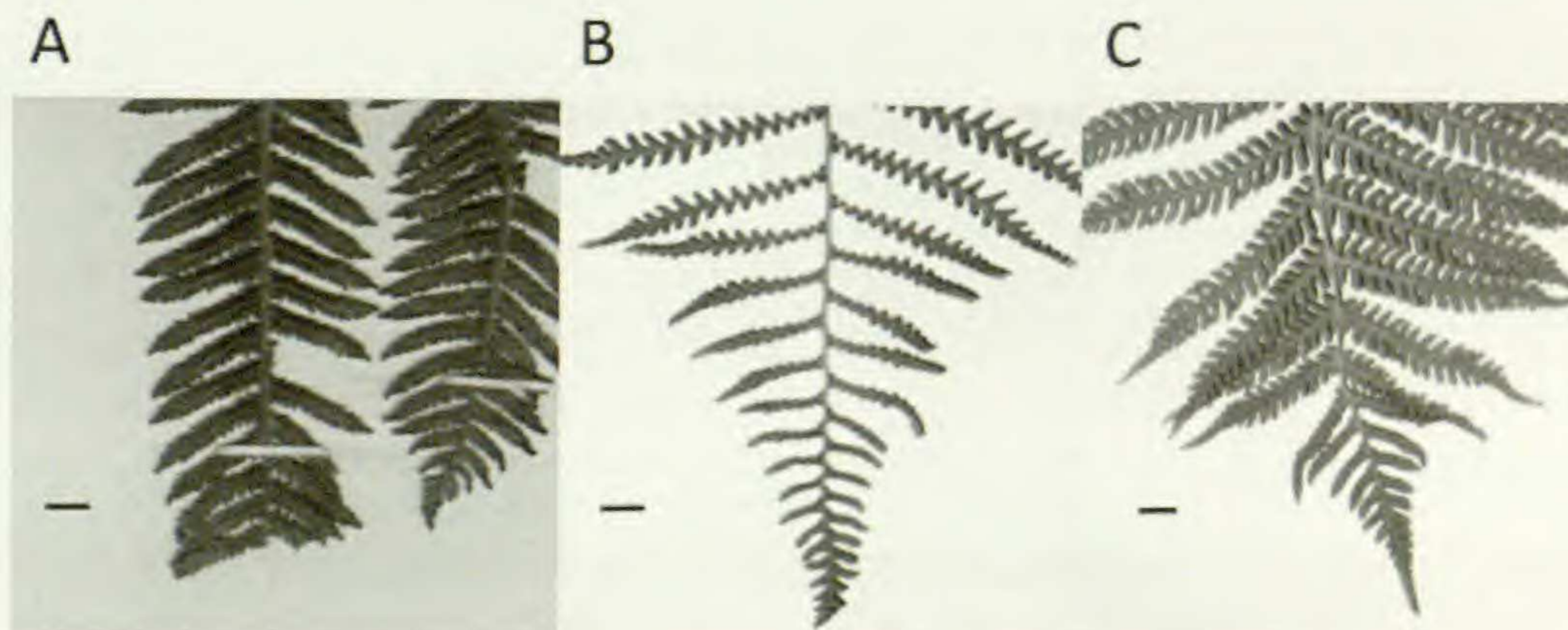


FIG. 1. A comparison of the pinnae. A: *Sphaeropteris tomentosissima*; B: Highland Lace; C: *Sphaeropteris cooperi*. The bars represent 1 cm.

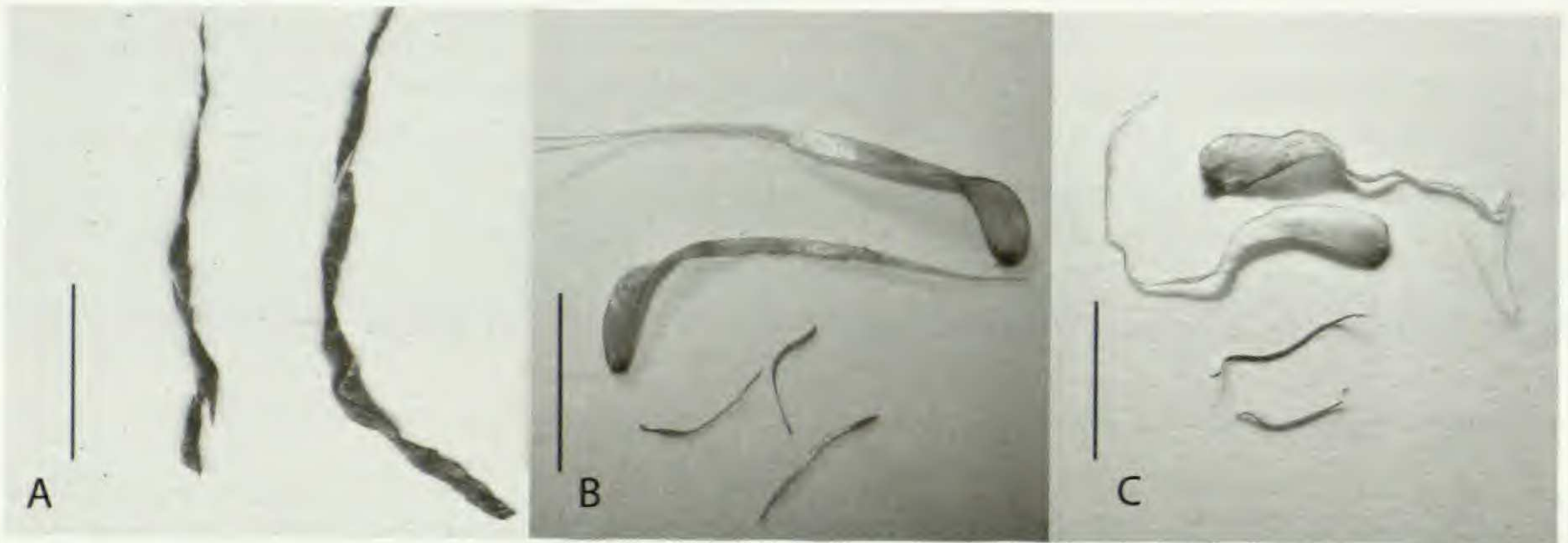


FIG. 2. A comparison of the stipe scales of *Sphaeropteris tomentosissima*, Highland Lace, and *Sphaeropteris cooperi*. A: Scales of *S. tomentosissima* are brown and twisted. B and C: Highland Lace and *S. cooperi* respectively both have large pale scales with dark setae on the edge as well as smaller narrow dark-red scales. The bars represent 1 cm.

fern *Sphaeropteris cooperi* despite their distinct differences in pinnae and leaf shape (Fig. 1).

The scales of Highland Lace were compared to *Sphaeropteris cooperi* and *Sphaeropteris tomentosissima*. The *S. tomentosissima* stipe scales are brown, twisted, and have edges bearing setae of the same color as the scales. In contrast, the broader stipe scales of Highland Lace and *S. cooperi* are whitish to light tan, with their margins usually bearing a very narrow row of dark reddish brown marginal cells and setae of the same color. Additionally, Highland Lace and *S. cooperi* have small narrow dark reddish brown scales on the stipe, which are absent on *S. tomentosissima* (Fig. 2A–C). Also particularly noticeable on *S. tomentosissima* are the very dense mats of small woolly scales on the abaxial side of all rachises (Fig. 3A–C), which are not present on *S. cooperi* or Highland Lace. The comparison of scales alone is highly suggestive that Highland Lace is much more closely related to *S. cooperi* than to *S. tomentosissima*.

In order to ascertain if Highland Lace is possibly *Sphaeropteris cooperi* and to further rule out *Sphaeropteris tomentosissima*, we compared *rbcL* DNA



FIG. 3. A comparison of the costae of *Sphaeropteris tomentosissima*, Highland Lace and *Sphaeropteris cooperi*. A: *S. tomentosissima* has a dense mat of small woolly scales on the abaxial side. B and C: Highland Lace and *S. cooperi* respectively do not have the dense mat of woolly scales.

TABLE 3. Dissimilarity matrix indicating the number of base-pair changes observed for the three loci, *rbcL*, *atpA* and *trnL* intron. Numbers in parenthesis indicate the total base pairs compared.

		Highland Lace	Cultivated <i>S. cooperi</i>	Flecker <i>S. cooperi</i>	<i>S. tomentosissima</i>
<i>S. tomentosissima</i>	<i>rbcL</i>	4 (1309)	4 (1309)	4 (1309)	-
	<i>atpA</i>	1 (1514)	1 (1514)	1 (1514)	-
	<i>trnL</i>	2 (554)	2 (554)	2 (554)	-
Flecker <i>S. cooperi</i>	<i>rbcL</i>	0 (1428)	0 (1428)	-	
	<i>atpA</i>	0 (1521)	0 (1521)	-	
	<i>trnL</i>	0 (554)	0 (554)	-	
Cultivated <i>S. cooperi</i>	<i>rbcL</i>	0 (1428)	-		
	<i>atpA</i>	0 (1521)	-		
	<i>trnL</i>	0 (554)	-		
Highland Lace	<i>rbcL</i>	-			
	<i>atpA</i>	-			
	<i>trnL</i>	-			

sequence data for both species (Newmaster *et al.*, 2006; Korall *et al.*, 2007). The Highland Lace sequence differed from the partial gene sequence of *S. tomentosissima* in GenBank by four changes over the 1309 base pair (bp) length, further evidence that they were different species (Table 3). This sequence was then searched on GenBank and surprisingly the top BLAST match for Highland Lace was a sequence from *Sphaeropteris excelsa* rather than the expected *S. cooperi*. The 1309 bp sequence of *S. excelsa* (AM410213) was identical to that portion in Highland Lace, while the *S. cooperi* sequence (SCU05944) differed by four changes over 1320 bp.

This *rbcL* sequence comparison seemed to indicate that Highland Lace was closer to *S. excelsa* than to either *S. cooperi* or *S. tomentosissima*. However, the scales on Highland Lace did not match this conclusion. Highland Lace and *S. cooperi* have both broad pale scales as well as small narrow dark red scales on its stipe, while *S. excelsa* has only broad pale scales. Additionally, Highland Lace (and *S. cooperi*) has small narrow dark red scales on its costa and costule while *S. excelsa* has a mat of whitish scales and hairs (Hoshizaki and Yansura, 2005). Since the scales on Highland Lace matched those of *S. cooperi* rather than *S. excelsa*, we decided to obtain additional *S. cooperi rbcL* sequence data from a cultivated plant and from four plants from the Flecker Botanical Gardens (Table 1). All five sequences were identical over the complete *rbcL* gene of 1428 bp, and these were exact matches for the Highland Lace gene.

In order to further confirm the identity of Highland Lace, the chloroplast *atpA* gene sequence (Schuettpetz *et al.*, 2006) was obtained from this plant, from the cultivated *S. cooperi* plant and from the four tree ferns in the Flecker Botanical Garden. All six sequences matched perfectly over the complete gene sequence of 1521 bp, while the GenBank partial sequence for *Sphaeropteris tomentosissima* differed by one change over 1514 bp (Table 3), resulting in one

amino acid change (T213N). There were no reference *atpA* sequences in GenBank for *S. cooperi* or *S. excelsa*.

As a final step, we obtained DNA sequences for the *trnL* (UAA) intron (Taberlet *et al.*, 2007). Highland Lace perfectly matched that of cultivated *S. cooperi* and the four Flecker Botanical Gardens specimens over the intron's 554 bp (Table 3), and these sequences also matched exactly the partially overlapping 534 bp of *S. cooperi* in GenBank (EU554328) and *S. excelsa* over 525 bp (AM410341). The *S. tomentosissima* sequence in GenBank differed by two bp over the complete 554 bp overlap (Table 3).

Sphaeropteris excelsa and *S. cooperi* are closely related (Tryon and Tryon, 1959; Tryon, 1970; Jones and Clemesha, 1981) and share at least partial common *rbcL* and *trnL* (UAA) intron DNA sequences. These *S. excelsa* sequences were subsequently reconfirmed using leaf material from a cultivated plant (Hoshizaki and Yansura, 2005). The phylogenetic relationship between these species is unknown, but less conserved non-coding (Shaw *et al.*, 2005; Kress and Erickson, 2007) or nuclear sequences (Sang, 2002) could resolve this question.

DISCUSSION

The identification of tree ferns is especially difficult when the country of origin is not known (Pryer *et al.*, 2010). While Australia has only eleven native species (Jones and Clemesha, 1980), the possibility of non-native spore arriving from nearby New Guinea or from the collections of tree fern enthusiasts within the country is certainly reasonable. The unique appearance of Highland Lace, in particular the reduced pinnules, almost certainly led Rod Hill to identify it as the non-Australian species *S. tomentosissima*. Upon re-examining Highland Lace, the traditional use of stipe scales for tree fern identification suggested that this identity was incorrect.

The more recently developed approach of using chloroplast or nuclear DNA sequences as barcodes for species identification (Kress *et al.*, 2005; Chase *et al.*, 2005; CBOL Plant Working Group, 2009) has been shown to complement traditional analyses based on morphological characters. While DNA sequence analysis is becoming a more widely used tool for this purpose, the public database is still somewhat limited in terms of species coverage. There are only about 150 *rbcL* sequences from *Sphaeropteris*, *Cyathea* and *Alsophila* in GenBank, while worldwide there are over 600 Cyatheaceae tree fern species (Large and Braggins, 2004). However, an enlarged DNA database will eventually provide a more robust system.

The confirmation that Highland Lace is *S. cooperi* required the use of both morphological characters and DNA sequence analysis. The early study of stipe scales showed that Highland Lace was not *Sphaeropteris tomentosissima*, but it did not demonstrate that it was *S. cooperi*. To do so was more tenuous considering that there are approximately 120 *Sphaeropteris* species worldwide (Large and Braggins, 2004), many with similar scale morphologies.

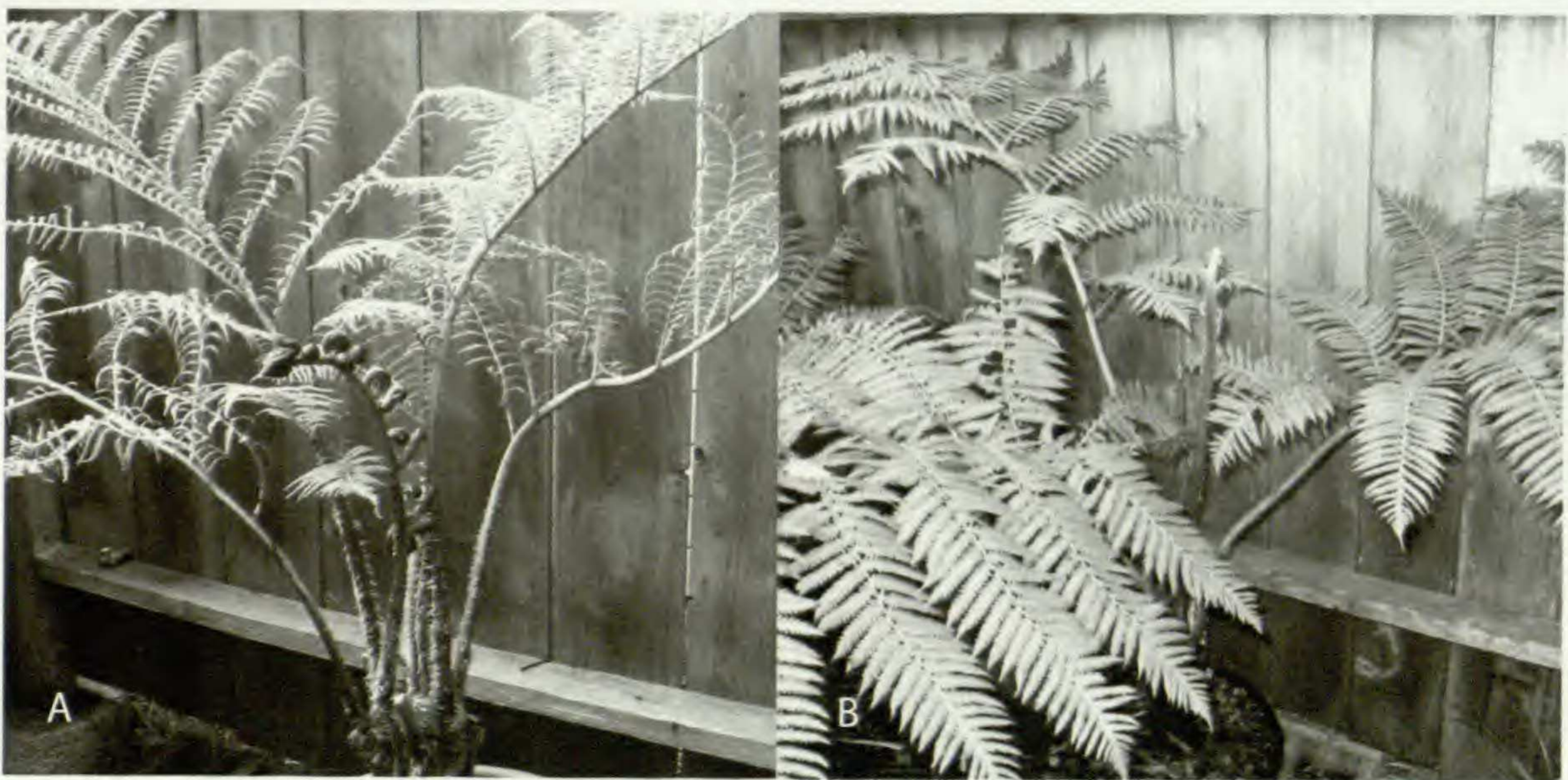


FIG. 4. An overall view of the tree ferns Highland Lace (A) and "Wild-type" *Sphaeropteris cooperi* (B) showing the significant differences in their general appearance.

Our first DNA sequence analysis based on *rbcL* confirmed that Highland Lace was not *S. tomentosissima*, but the effort to determine if it was related to *S. cooperi* resulted in the discovery of a GenBank voucher that was misidentified (see Results for details). As a result, new reference sequences were made for *S. cooperi*, which all proved identical to the Highland Lace sequence. Further DNA sequence analysis based on the chloroplast *atpA* gene and the *trnL* (UAA) intron also confirmed that Highland Lace is *S. cooperi* (Table 3).

As a practical way to identify a tree fern species, DNA barcoding is an important tool, but with the limited data available, it cannot be used exclusively. The initial Highland Lace *rbcL* sequence quickly showed that this tree fern was not *S. tomentosissima*. However, given the sequences that currently exist in GenBank, DNA barcoding could not distinguish whether *S. excelsa* or *S. cooperi* was the correct species. Morphologically specific features, particularly the leaf scales in tree ferns, still play an important role in fern identification. The use of morphological characters that initiated this investigation later led to the discovery of the error in the database and its subsequent correction, and scale characteristics ultimately allowed us to choose *S. cooperi* as the correct species. The interplay of these two methods was important throughout this study.

At first glance, it is difficult to think that Highland Lace and *S. cooperi* are actually the same species because their general appearances are so strikingly different (Fig. 4). *Sphaeropteris cooperi* is native to eastern coastal Australia and is known to be variable in form (producing cultivars including Brentwood, Robusta, Allyn Lace, and Allyn Kiest). Most of these variants, however, are quite modest compared to what is observed in Highland Lace with its conspicuously contracted, recurved margins and the reduced size of the

pinnules. *Sphaeropteris cooperi* shares this ability to produce multiple variants with a limited number of other ferns. Species such as *Athyrium filix-femina* (L.) Roth and *Polystichum setiferum* (Forssk.) Moore ex Woyнар are also known to produce many variants that have contracted or reduced blade surfaces, recurved margins, and smaller dimensions, plus many more deviations from the typical shape (Rickard, 2000; Hoshizaki and Moran, 2001).

A search of the literature suggests that this unusual tree fern may have been reported earlier. A description of *Sphaeropteris cooperi* (in Flora of Australia, 1998) mentions the existence of an unnamed narrow pinnule variant:

“An occasionally cultivated form of *Cyathea (Sphaeropteris) cooperi* from central and northern Queensland has narrow recurved abaxially glaucous pinnule lobes, with the majority of rhizome and stipe scales lacking any brown coloration. The sori in this form are commonly restricted to the basal part of each pinnule, at least on younger plants. The Victorian collection may be an isolated accidental occurrence rather than a sample from a naturalized population.”

This is possibly the same plant as Highland Lace. However, in the Flora of Australia description, the stem and stipe scales of this fern are said to lack any brown coloration while the Highland Lace specimens in the US have dark red-brown margins, bearing setae. The sori on Highland Lace also may extend well beyond the basal part of each pinnule to near the tip in the US specimens. If Rod Hill’s website is correct concerning the origin of this unusual fern in a spore pan (but in New South Wales instead of Queensland), we may consider this form an accidental occurrence. However it cannot be ruled out that this aberrant plant may also exist in the wild.

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